AN EXPERIMENTAL STUDY OF THE STREPTOCOCCI FOUND IN PYORRHOEA ALVEOLARIS.

by

MURIEL J. THOMSON, M.B., Ch.B.

Thesis for the Degree of M.D.

AN EXPERIMENTAL STUDY OF THE STREPTOCOCCI FOUND IN PYORRHOEA ALVEOLARIS.

INTRODUCTION.

There is great diversity of opinion with regard to the probable relationship of pyorrhoea alveolaris to secondary lesions associated with it.

There is an irrational tendency on the part of the medical profession today to advise extraction of teeth in such cases. The main object of this work was to throw some light on the advisability of such a drastic procedure.

The investigation has covered a period of 2 years. The material was obtained from patients in the medical wards of the Royal Infirmary, Edinburgh, care being taken to choose patients, who showed some other organic lesion special regard being given to rheumatoid arthritis, endocarditis, bronchitis and arteriosclerosis. The clinical history and symptoms of the secondary condition were most carefully ascertained.

In the Wards of the Royal Infirmary, especially those of Professor Bramwell and Dr Chalmers Watson I was given every facility to study the case histories of/

of the patients from whom I obtained the pathological material. Mr Gibbs also gave me valuable help in my investigation. I obtained some of the material from Dental Out-Patients under his care, but the patients frequenting this department did not always show other lesions, and suitable material was but rarely available.

After obtaining the material, it was examined by inoculation into mice and culturally, for pneumococci, streptococci and micrococcus catarrhalis. Streptococci, which were the only organisms found present, were then typed according to Holman's classification. Pure strains of Streptococci were inoculated intravenously into rabbits to ascertain whether they produced lesions similar to the secondary ones in the patients, or whether no such relationship existed.

The question of such great practical importance, as well as interest is:

Can any of the forms of arthritis, endocarditis, arteriosclerosis and nephritis, which up to the present have been regarded as "Idiopathic" conditions, be secondary to a pre-existing infection of the periodontal membrane and if so is it due to a diffusion of toxin from the Streptococci or to their actual presence?

A FEW NOTES ON THE CLINICAL ASPECT OF PYORRHOEA ALVEOLARIS.

Pyorrhoea Alveolaris is no new clinical entity, as can be definitely proved by the examination of old skulls.

It occurs in domestic animals as well as human beings.

Most observers agree that Pyorrhoea is due to several factors, e.g.

(1) The resistance of the gum is in all probability decreased by the drying up of the saliva in mouth-breathers, especially around the bases of the incisor teeth.

(2) Some writers regard incorrect diet as an additional factor. This is a possibility when one regards the condition of the gums in certain diet deficiency diseases e.g. scurvy.

(3) A few regard the condition as a secondary effect due to a general lowering of the patients' resistance.

(4) It may be due to the lodging of tartar or calculus in the gum recess. The hard material gradually increases in amount about the neck of the/

the tooth; the gum and periodontal membrane gradually become more and more irritated, micro-organisms accumulate and an inflammation of the part commences.

It is a progressive condition.

Pyorrhoea alveolaris is not a good term to apply to the disease, for the presence of an actual purulent discharge is not essential to the condition, although it is usually present.

The lesion begins as a gingivitis. The gum usually becomes swollen and somewhat spongy, and in addition it may be either red or less frequently pale in colour. The gum may commence to retract from the tooth socket at this stage and associated with this, as a frequent early sign is a slight bleeding.

As the condition advances the veins, in relation to the affected tooth or teeth, become engorged and the gums tend to fall away more and more from the teeth, thus forming a definite pocket.

What really occurs is that the depth of the pocket increases pari passu with the proliferation of the lining epithelium. Once the epithelium has been separated from the tooth, it cannot again become attached, so that the pocket becomes a permanent structure.

Various other views are presented with regard to the/

the formation of the pocket.

(1) That the alveolar process disappears, thus a space is formed and the epithelium is permitted to grow down along the root of the tooth; or

(2) That the root becomes necrotic and is treated like a foreign body and the epithelium from the neck of the tooth grows down to enclose it; or

(3) That the proliferation and in growth of the epithelium is due to some inflammatory stimulus.

As soon as there is the smallest lesion of the epithelium, lymphoid nodes make their appearance in the gum. The increase in these is equal to the increase in epithelial tissue. Both lymphoid nodes and epithelium proliferate as the result of a common stimulus. The condition may advance so far as to involve the whole tooth socket. The teeth may become loose and even drop out.

FORMS OF THE DISEASE.

There are 2 forms which are present in practically every case of pyorrhoea alveolaris.

1. The chronic form, and
2. The acute form.

The chronic form may be regarded as a very slowly progressive disease which is liable to acute exacerbations at longer or shorter intervals, depending chiefly on the general health of the patient. In the latter form there is usually a large amount of purulent discharge, a foetid breath and often looseness of the teeth.

In the series of cases of pyorrhoea alveolaris examined, three cases (which includes a case of acute alveolar abscess) were in the acute phase while the remaining 8 cases were in the chronic phase of the disease.

AGE INCIDENCE:

The condition occurs chiefly between 30 and 50 years of age.

According to Euler[1] the incidence of Pyorrhoea Alveolaris has greatly increased since the war. This may or may not be the case. In the first place, there are not sufficient statistics by which this can be definitely proved and in the second, the attention of most medical men and of the general public has been increasingly directed towards this condition by publications and advertisements, especially in the lay press.

Pyorrhoea itself is of little danger but its effects may be far reaching.

PART I

THE BACTERIOLOGY OF PYORRHOEA ALVEOLARIS.

ORGANISMS FOUND IN PYORRHOEA ALVEOLARIS.

It has been well recognised that this condition is associated with a somewhat varied flora. Streptococci, Staphylococci, Pneumococci, Bacillus fusiformis and Leptothrix have all been found present. Attention has been directed to the presence of amoebae - the entamoeba gingivalis - this organism has been regarded as the etiological factor by some.

Bertand and Valadier[2] found Streptococci in the pus and these always in large numbers in cases of Pyorrhoea Alveolaris. In most cases they found other associated organisms, among these were the micrococcus catarrhalis and the pneumococcus.

Gilmer and Moody[3] found that Streptococci were the organisms most constantly present in Pyorrhoea Alveolaris.

Hartzell and Henrici[4] also showed that Streptococci of the viridansgroup were constantly present in peridental suppurative lesions. They examined 162 cases of pyorrhoea alveolaris and apical abscess and found/

found Streptococci in 150 cases. These belonged to the Mitis, Salivarius and Faecalis groups. They found other organisms present in the cultures in a fair proportion of cases.

Rosenow has also isolated Streptococci from cases of Pyorrhoea alveolaris.

NORMAL FLORA OF THE BUCCAL CAVITY.

Before going any further it would be well to mention that the normal flora of the mouth has been investigated by many workers, and that Streptococci of the viridans type have been found.

Seitz[5] describes the occurrence of Streptococci belonging to the viridans type in the mouth.

On the whole Streptococci of the Viridans group, are almost always present in the normal mouth. The haemolytic varieties are less frequent. Owing to the presence of these Streptococci normally, one must be very certain that a condition such as pyorrhoea exists, and, that all the organisms on the gingival mucous membrane are removed before withdrawing pus from the pyorrhoeal pockets.

THE CLASSIFICATION OF STREPTOCOCCI.

The question of adopting a classification presented a grave problem. There is so much diversity of opinion as to whether to rely on the older methods of classification based on power of haemolysis, sugar reaction tests and bile solubility or to adopt the more recent serological methods of classification.

A review of the literature did not justify the application of a classification based on serological methods as far as non-haemolytic Streptococci were concerned. In fact the classification is very unsatisfactory.

LITERATURE ON SEROLOGICAL CLASSIFICATION OF STREPTOCOCCI.

A. By Compliment Fixation.

Kinsella and Swift[6] examined non-haemolytic Streptococci for specificity of compliment fixation. They found that there was a definite relationship in their results, but that, while in some reactions the antigen was specific for one serum, in others the serum was fixed by several antigens.

Howell[7] stated that the positive fixations could not/

not be grouped in any way that would justify a classification of Streptococci based on the complement fixation. She also found that non-haemolytic antisera apparently gave even less specific complement fixations than haemolytic antisera and also that there is no relation between complement fixation tests and groups based on the fermentation of carbohydrates.

Aschner[8] obtained marked fixation with a homologous antigen, and a positive, although less marked fixation with a mixed antigen.

B. By Agglutination.

Many reliable workers have endeavoured to correlate the various cultural relationships of Streptococci with agglutination - among the workers on this have been Besredka[9], Floyd and Wolbach[10], Hiss[11] and Krumwiede and Valentine[12], - the work has been met with little success.

Swift and Thro[13] found specific reactions for group classification of Streptococci, - but they did not find specific strains by complement fixation and conglutination.

Wolbach[14] obtained promising results when he compared his fermentation and agglutination reactions. He found that although the reactions were not specific enough for classification, the present methods of classification might be simplified by/

by the aid of agglutination tests.

Havens[15] in his classification found that haemolytic streptococci could be differentiated by agglutination tests, bactericidal experiments and the protective properties for mice. He produced a serum high in agglutinins in rabbits and found that in a series of 110 rabbits 55% fell into one group.

Kligler[16] believes that agglutination tests cannot be relied on for the classification of Streptococci.

Smith and Brown[17], on the other hand, find this a useful method, although the groups of haemolytic Streptococci do not appear to be as clear cut as those into which the pneumococcus falls.

Much work is still required to throw light on this method of classification.

C. By the Precipitin Test.

Barnes[18] found that precipitins produced by immunising rabbits each with one of several strains of Streptococci classified by their haemolytic and fermentation reactions, are relatively specific in high dilutions, but also give group reactions, usually, however, in low dilutions. The precipitin reaction agrees with the haemolytic and fermentative reactions in classifying streptococci.

PART II

BIOLOGICAL CLASSIFICATION

Owing to the many difficulties and lack of conformity in the classification of Streptococci according to serological methods, I adopted the classification according to the biochemical tests, recommended by Holman[19].

A classification of Streptococci on such a basis is too clear cut and artificial to be of any permanent value in the classification of Streptococci. In the present poor state of our knowledge, although artificial, it appears to be the only method of classification by which results obtained by the investigations of Streptococci, similar to the present one, can be passed on to other workers, so that they can be more or less interpreted by them.

Much has been said and written concerning the lack of constancy in the carbohydrate reactions obtained with streptococci. Even in the few cases I have examined I have found this. Rabbit 8 was injected with a pure strain of organism reacting to the Salivarius variety of Streptococci. On regaining organisms from this rabbit the reactions given by the strain on being re-examined conformed with those of the/

the Mitis variety.

Gordon's[20] classification was too elaborate a method to adopt in the present investigation. His method included 9 different tests, which results in the differentiation of so many types, that it is not only confusing, but of little practical value.

Andrewes and Horder[21] find there is a remarkable constancy in the carbo-hydrate fermentation reactions for any one strain of streptococcus.

Holman's classification consists in the division of streptococci into 2 large groups, viz:-

I Haemolytic Strains

II Non-haemolytic Strains.

There are 8 subdivisions of each of these groups depending on their action on mannite, salicin and lactose. He thus divides all Streptococci into 16 groups. (See Tables I and II.

TABLE I.

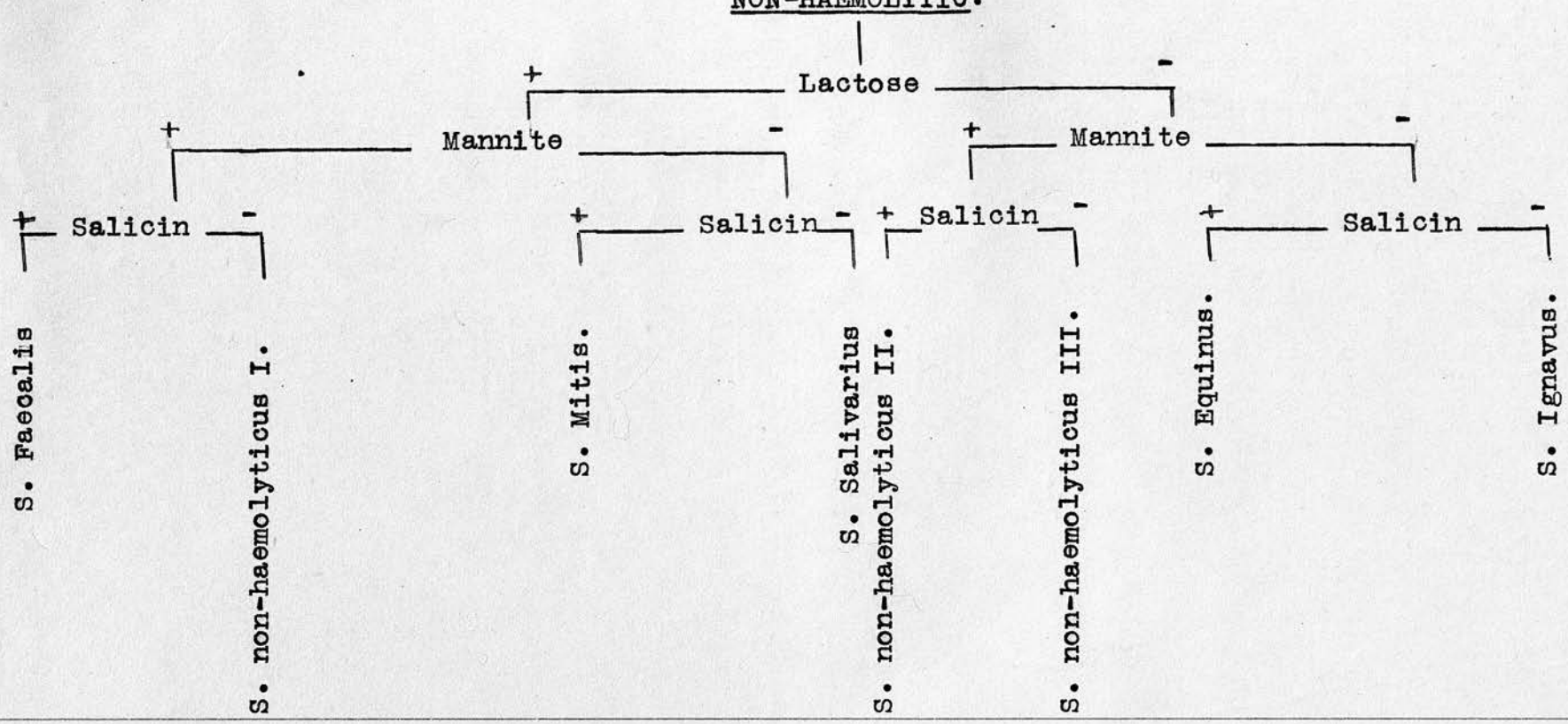
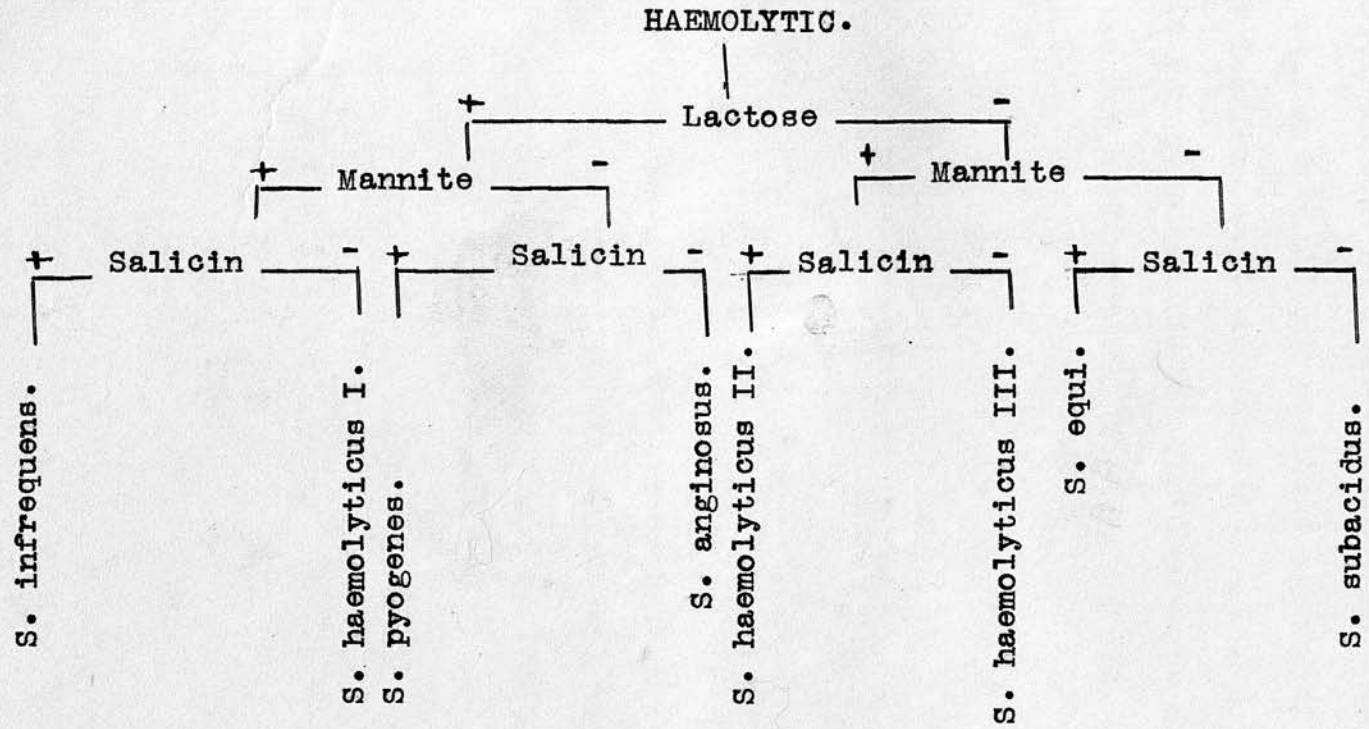

NON-HAEMOLYTIC.
Lactose
+
-
Mannite
+
-
Mannite
+
-
Salicin
+
-
Salicin
+
-
Salicin
+
-
Salicin
+
-
S. Faecalis
S. non-haemolyticus I.
S. Mitis.
S. Salivarius
S. non-haemolyticus II.
S. non-haemolyticus III.
S. Equinus.
S. Ignavus.

TABLE II.


HAEMOLYTIC.
Lactose
+
-
Mannite
+
-
Mannite
+
-
Salicin
+
-
Salicin
+
-
Salicin
+
-
Salicin
+
-
S. infrequens.
S. haemolyticus I.
S. pyogenes.
S. anginosus.
S. haemolyticus II.
S. haemolyticus III.
S. equi.
S. subacidus.

Holman's method of classification proved the most practical one from every point of consideration for the type of investigation carried out.

This investigation embraced a study of only Streptococci present in the condition, as they were regarded as the most likely positive agents of secondary lesions, e.g. endocarditis. It is well known that Streptococci of the viridans type are responsible for endocarditis, nephritis, etc.

A SHORT NOTE ON THE CASES STUDIED.

The cases from which the pus was taken was carefully chosen, as one wished only to investigate those in which there were definite secondary lesions, which might be attributed to the pyorrhoeal condition.

A summary of these will be found in Table III.

TABLE III.

TABLE III.

Case	Strain	Stage of Pyorrhoea	Associated pathological conditions	Types of Streptococci
1	A	Acute	Lombar pains and constipation	S. Faecalis
2	B	Chronic	Herpetic eruption - pain in left arm, side of neck and chest.	S. Salivarius
3	C	Chronic	Pain, stiffness and swelling of all joints.	S. Mitis
4	D	Acute	Bronchitis and emphysema.	S. Faecalis S. Mitis
(5	E	Acute Alveolar Abscess	-	S. Mitis)
6	G	Chronic	Arteriosclerosis and high blood pressure.	S. Salivarius
7	H	Chronic	Bronchitis and polyuria.	S. Mitis
8	J	Chronic	Neuritis and fibrositis.	S. Salivarius.
(9	K	Acute	Pus from abscess in neck of child.	S. Faecalis)
10	L	Chronic	General weakness and debility. Swelling of knee.	S. Faecalis
(11	O	Acute	Osteomyelitis.	S. Infrequens)
12	P	Chronic	Pain and swelling affecting several Joints.	S. Faecalis
13		Chronic	Toxic Jaundice	S. Salivarius

METHOD OF TAKING THE PUS FROM THE PYORRHOEAL POCKETS.

In order to ensure against contamination by mouth organisms, the patients first rinsed their mouths thoroughly with a solution of Eusol. The mouth was then kept open and the parts from which the pus was to be taken, were well swabbed with absolute alcohol over a wide area. A sterile platinum loop was then inserted into the pocket and gently pushed down as far as it would go. The loopful of pus was then transferred to a tube containing 1cc of sterile normal saline. This procedure was repeated 3 times, two loopfuls being inserted into one tube of saline, while the remaining loopful was transferred to a second tube.

This method of procedure was adopted in all but 2 cases in which the teeth were extracted. In these cases the roots were snipped off and shaken up in 2ccs. of normal saline.

The specimens were taken from only one tooth in each case, and that showing the most marked pyorrhoea.

TESTS FOR VIRULENCE AND PRESENCE OF PNEUMOCOCCI.

The entire contents of the tubes containing the two loopsful of pus were injected directly into the peritoneal/

peritoneal cavity of a mouse in order to test the virulence of the organism under investigation, and, at the same time to find out whether there were any pneumococci present.

As is well known, white mice are highly susceptible to pneumococci and after inoculation die within a day or two, of septicaemia with typical capsulated diplococci in the heart blood. In none of these experiments was this finding observed. Subsequent cultural tests showed the same. All the mice inoculated were killed about a month or 6 weeks after injection. They never showed the slightest symptoms.

1cc. of the emulsion made from the pus adhering to the roots of the extracted teeth was also injected intraperitoneally into mice, but with the same negative results.

ISOLATION OF STREPTOCOCCI.

A loopful of the emulsion of the second tube, and, in the case of the extracted teeth, of the remaining cc. was inoculated on to three serum or blood agar plates and incubated for 18 to 24 hours.

In every case a pure growth of streptococci was obtained. The fact that cultures invariably yielded a pure growth of streptococci is specially noteworthy.

The/

The technique adopted for obtaining the pus from the pockets eliminated salivary and mouth organisms. Streptococci therefore were the only aerobic organisms culturable by the ordinary methods, for pneumococci were not demonstrable by growing on serum or blood media or by the inoculation of mice.

Three different colonies of Streptococci were transferred from one of the original plates into three different tubes of serum or blood broth and incubated at 37° for 18-24 hours.

An agar plate was then inoculated from each of the above mentioned tubes and incubated overnight. A single colony was picked off each plate, a blood or serum broth tube was again inoculated and placed in the incubator overnight. A further plate was inoculated with the organisms from each tube and incubated. Finally a colony was picked off each plate and inoculated into serum broth and incubated.

This procedure was devised in order to obviate any dubiety on the purity of the cultures used.

The 3 pure cultures obtained in this way were typed according to Holman's classification. With one exception the 3 strains derived from each case proved similar in type.

TESTS EMPLOYED IN THE CLASSIFICATION OF STREPTOCOCCI.

I. BILE SOLUBILITY TEST.

This is the only reliable test for the differentiation between Streptococci and pneumococci. A plain nutrient broth culture of the organisms was incubated for about 18 hours when an equal amount of a 5% solution of sodium taurocholate in normal saline was added. The mixture was well shaken and allowed to stand at room temperature for at least 1½ hours. The organisms were always found to be insoluble.

Absence of lysis of the organisms was always determined microscopically, except in a few cases where the mixture had to be plated, on account of the scanty growth.

Neufeld[22] noticed that if he added 0•1cc. of normal rabbits bile to every 1 or 2ccs. of a pneumococcal broth culture the pneumococci lysed. The Streptococci were not in any way affected by the addition of bile.

Libman and Rosenthal[23] have found this differentiation most reliable, while Levy found that sodium taurocholate dissolves the pneumococcus and the Streptococcus mucosus grown in broth but not other streptococci.

II. HAEMOLYTIC TEST.

The haemolytic test was carried out in every case by the method described by Sekiguchi[24] which eliminated the personal factor which is present when employing blood agar plates as were originally used by Schottmuller[25].

The test on blood agar plates was employed in addition in almost every strain investigated, as an additional guard.

The following steps were carried out in the haemolytic test.

Two tubes, each containing 0·6cc. of nutrient broth and 0·6cc. of sheep's serum, were heavily inoculated with the strain of Streptococci to be examined and placed in the incubator at 37°C. for 16-18 hours in order to obtain the maximum content of haemolysin, as some strains show a reduction in haemolysin after 18 hours incubator.

For testing the first 6 strains, thrice washed defibrinated corpuscles were used; they were made up to the original volume of whole blood.

Every test was performed in duplicate.

3·8ccs. of normal saline were put into a tube, which contained 1cc. of the supernatant fluid from the centrifuged 16-18 hour culture of streptococci. To this 0·2cc. of the prepared rabbit's blood was added. The mixture was then well shaken and placed in the incubator/

incubator for 2 hours at 37°C. After this the tubes were placed in the ice chest and the readings were taken the following morning.

A control tube containing 3·8ccs. of normal saline and 0·2cc. of the defibrinated washed rabbit's blood was put up with each experiment.

One cc. of the supernatant fluid off the cultures was used to avoid missing weak positive results.

None of the organisms obtained from cases of pyorrhoea alveolaris showed the slightest trace of haemolysing rabbit's blood, either on blood agar plates or by the method described.

To control this method of investigation two strains of Streptococci, known to be haemolytic were also tested; they showed complete haemolysis.

In the last 5 strains tested the rabbits blood was defibrinated, but not washed.

All the cultures used for the haemolytic test were examined microscopically - an abundant growth of streptococci was always found.

In view of the observation made by Lyall[26] and Cowan[27] I tested different layers of culture fluid and with negative results in all cases.

III. CARBO-HYDRATE FERMENTATION TESTS.

The sugars used were those indicated by Holman[19]. They/

They were as follows:-

Inulin: A polysaccharide.

Salicin: A glucoside.

Mannite: A hexahydric alcohol.

Lactose: A disaccharide.

Only one of these strains was found to ferment inulin and that very slightly, namely strain "G". In order to be sure of the purity of the inulin used against this strain, it was carefully washed in water, to dissolve the fructose. Strain "G" was again tested against the washed inulin, but still a slight fermentation occurred. The sugars were in a 1% solution in Hiss's serum water which was made up with one part of water and two parts of serum. The indicator used was litmus.

There was only one example of a lack of constancy in these carbohydrate tests and that was in rabbit 9, which although injected with S. Salivarius, revealed S. Mitis in the cerebro-spinal fluid after death.

In all other cases, when organisms were recovered after animal inoculation, there was no variation from the original reactions to the sugars.

It was found that all the tubes had to be heavily inoculated in order to be able to place any reliance on the tests. In a few cases fermentation did not occur until about the fourth or fifth day of incubation.

If/

If the tubes for the carbohydrate fermentation tests were only inoculated with a few drops of an 18 hour serum broth or brain broth culture, fermentation did often not take place, which if ½cc. or more was used to inoculate each tube with, fermentation might occur.

Owing to these facts reliance was only placed on negative results in tubes which had been inoculated with ½cc. or more of an 18 hour serum broth culture and which had been incubated at 37°C. for not less than one week.

Lyall[26] found the reactions he obtained with Hiss's mannite and inulin were sufficiently definite to warrant a tentative classification based on the reactions of these. In the series of organisms at present under consideration, using lactose instead of raffinose, one was able to support Lyall.

Kendal, Day, Walker and Ryan[28] while working together on the fermentation reactions of certain Streptococci, found that carbohydrates possess value in the classification of bacteria through the fact that a definite relationship apparently exists between the stereo-configuration of various definite groups of these substances and the ability of the organisms to ferment them. They also speak of the unerring specificity of these reactions, so that it is evident from what they found that they do not believe in/

in inconstancy of the reactions in even a small percentage of the organisms.

Andrewes and Horder[21] found that by using Gordon's classification (Holman's classification is a modification of it) the reactions were remarkably constant for any given strain of streptococcus, although they found some exceptions.

Ainley Walker[29] supported the evidence that sugar reactions were unreliable in the differentiation of Streptococci and that the variability is largely dependant on the immediate previous environment of the organisms. He draws attention to the remarkable degree of constancy in the organisms freshly isolated from the buccal cavity, for it is most unlikely that all the streptococci which enter the mouth conform to one type. He lays great stress on environment.

Aschner[8] gives a very large percentage of non-haemolytic streptococci fermenting inulin. He found that in 12 strains, 5 fermented inulin. Three of these strains showed variability of their reaction to inulin on re-testing.

Although much can be said in favour of Holman's classification, there is no relationship between the various types of non-haemolytic streptococci and the pathological conditions produced by them.

Films for microscopical examination were made from every tube put up for the carbohydrate tests after fermentation/

fermentation had occurred or after the completion of a week's incubation.

Morphologically pure streptococci were always found.

IV. MORPHOLOGY.

The variations in morphology are great, but no reliance can be placed on these for the classification of Streptococci. The appearances are often identical with those of the pneumococcus.

The following table gives a resumé of the reactions found in the various strains isolated.

TABLE/

TABLE

Strain:	Haemolyt. Reaction	Bile Solubil.	Inulin	Salic.	Lact.	Mannite
A.	-	Insol.	-	+	+	+
B	-	"	-	-	+	-
C	-	"	-	+	+	-
(D	-	"	-	+	+	+
(D	-	"	-	+	+	-
E	-	"	-	+	+	-
G	-	"	±	-	+	-
H	-	"	-	+	+	-
I	-	"	-	-	+	-
J	-	"	-	-	+	-
L	-	"	-	+	+	+
P	-	"	-	+	+	+

N.B. "E" was from a case of acute alveolar abscess.

From this table it can be seen that all the organisms were of the nonhaemolytic type. In all except one case, only one type of organism was found in each case. It is interesting to note that all the types conform with those which are normally found in the buccal cavity.

There was no association between the types of organisms obtained and the types of secondary lesions found in the patients, although the number of cases was really too small to place any reliance on this point.

The results of these observations show that:-

(1) Streptococci were constantly present;

(2) They belonged to the non-haemolytic group;

(3) They do not belong to our fermentative type, but include different ones, e.g. mitis, salivarius, and faecalis.

CONCLUSIONS.

1. By the technique used for obtaining pus from cases, pure growths of streptococci were obtained in all cases.

2. In no case was the pneomococcus demonstrated by culture or animal inoculation.

3. With one exception, the streptococci isolated were of one type (Holman's classification).

4. Streptococci were all non-haemolytic. There were 3 types present viz.: mitis, faecalis and salivarius.

5. Generally on retesting strains of streptococci, no inconstancy in their sugar fermentation reactions has been found.

PART II.

EXPERIMENTS WITH ANIMALS.

ANIMALS EMPLOYED.

1. MICE.

42 mice were used in this series of experiments. The main object in using mice as experimental animals was twofold:-

I. To test the virulence of the streptococci.

II. As a test for the presence of pneumococci.

It was found impossible to employ mice for any further purpose during this research on account of the presence of endemic mouse typhoid. The presence of this disease was especially evident when an attempt was made to regain the streptococci from the heart blood. Almost all the cultures in these cases were found to be heavily overgrown by an organism of the coli-typhoid group, which resembled the bacillus of Gärtner. The lesions found on post mortem examination conformed more to those which one would expect from the latter organism than to the Streptococci.

2. RABBITS.

49 rabbits were employed during the course of the/

the research and of these five were used as controls and four were inoculated with haemolytic streptococci from a case of osteomyelitis.

40 rabbits were used for the investigation of the strains of streptococci recovered from cases of pyorrhoea alveolaris.

Healthy tame rabbits were employed and their weights varied from 470 to 2400 grammes.

TECHNIQUE OF INJECTIONS.

In every case the injection was made intravenously and the marginal ear vein was selected for this purpose. At no time did the quantity injected exceed 10 ccs. and 4ccs. was the average amount utilised.

In all cases great care was taken to ensure the purity of the strains. This was ensured by the following procedure. The organisms were grown on serum or blood agar plates and replated twice in each case from a single colony. Finally the culture was transferred to serum broth in order to facilitate further manipulations.

Three different media were used during the further preparation of the organisms for injection and these were inoculated from the same serum broth culture mentioned above.

As/

As a final test of purity the suspension was examined microscopically before injection.

CLASSIFICATION OF EXPERIMENTAL ANIMALS.

The animals were subdivided into definite series according to the final method of growth.

Series A.

Organisms grown on serum agar slopes.

Series B.

Organisms grown in serum broth.

Series C.

Organisms grown on blood agar slopes.

Series D.

Organisms grown on serum agar followed by serum broth.

During the consideration of the above series the facts concerning each will be detailed in the following order:-

1. Cultures employed.
2. Alterations in weight.
3. Influence of passage on the virulence of the organisms.
4. Influence of the age of the strain on the virulence of the organisms.

5./

5. Lesions and symptoms produced.

6. Recovery of the organisms from the inoculated animals.

   (a) Technique.

   (b) Source from which the organisms have been regained.

7. Pathology of the lesions.

SERIES A.

1. Cultures employed.

Serum agar tubes were inoculated from the serum broth culture previously described. These tubes were then placed in an incubator at 37°C. for about 18 hours. At the end of this time the slopes were carefully examined to confirm the purity of the strain. The colonies were washed off and for this purpose about 1cc. of normal saline per tube was used. The washings were collected into a sterile tube and shaken until a uniform emulsion was produced. A film of the emulsion was examined microscopically as the final test of purity.

The number of slopes used for a single inoculation varied, but never exceeded 9 serum agar slopes.

4-8ccs of the emulsion were injected as has already been described and a careful record of the progress/

progress and weights of the animals concerned kept. A complete summary of these weights for all series is given in Table IV.

TABLE IV./

TABLE IV.

R = Rabbit.

The figure represents the strain, or, if derived from another animal its source with regard to fluid or tissue.

In this table the first row represents days of the year - (low number beginning after high ones are days from the following year).

The middle row represents the weights in grammes.

The lowest row shows the ~~bulk~~ dose injected derived from the number of B.A. (bl. agar), etc. tubes indicated.

R1 (G1)	118	119	121				
	2000	1800	1500				
	6SAS		killed				
R2 (G1)	110	115	118	119	121	123	124
	2400			2600		2050	2000
	6SAS	7SAS	7SAS		9SAS		died
R4 (CSF R2)		131	133				
		1770	1600				
		5SAS	died				
R5 (12)		131	138	144	148	155	158	159
		1450	1420	1370		1340	1250	1200
		5SAS		5SAS	5SAS		5SAS	died
R6 (CSF R4)		136	138	144	148	152	156	163
		1750	1740			1520	1410	1270
				5SAS	5SAS	5SAS	5SAS	died
R7 (J3)		156	158	163				
		1340	1170	1090				
		5SAS		died				

R8/

R8 (OSF R1)	144	152	153	156	163	170	172	178	181
	1640	1670	1620		1690	1800	1730	1690	
	5SAS	5SAS		5SAS	5SAS	5SAS			
	184	187							
	1770	1350							
	5SAS	killed							
R9 (G3)	134	138	144	148	153	155	156	163	
	1750	1870	1800		1650	1570	1540	1370	
	5SAS		5SAS	5SAS			5SAS	5SAS	
	170	171	172	177	184	185	188		
	1320	1280	1230	1150	1350	1390	1390		
	5SAS	5SAS			5SAS	5SAS	died		
R10 (OSF R9)	191	201	207						
	2030	1940	1850						
	2ccB	2ccB	killed						
R11 (OSF R10)		216	226						
		1370	1370						
		2cc5B	killed						
R13 (OSF R4)		180	180	199	212	276	281	283	
		2190	2320	2330	2460	2730	2520	2430	
		5SAS	5SAS	5B				killed	
R14 (OSF R5)		276	279	281	282	191			
		2190	2320	1488	1540	1670			
		4cc5B				killed			
R15		278	279						
		1600	1590						
			died						
R16 (OSF R5)		282	289	292	320	323	326	330	
		1770	1850	1950	1520	1340	1320	1260	
		4.25							
		5BA							
		341	345	351					
		1440	1420	1420					
		3cc5BA		killed					

R17/

R17 (12)	342	345	348	353	354	362
	970	840	740	710	750	820
	5cc4.6 BA					died

R18 (URINE R17)	12	14	15	16			
	1050	999	1030		17	19	
	3cc4.5 BA			3cc3BA	1010	1000 died	

R19 (URINE R17)	12	15	18	19	21			
	550	520	520	480	370			
	1.5cc 2.5BA			3cc3BA	died			

R20 (HB R17)	12	14	15	18	19	21	23	25
	480	500	500	510	530	490	460	3925
	2.25cc 4BA				.75cc 1.25BA			

R21 (LIVER R17)	12	14	15	18	19	21	23
	570	570	600	640	620	600	490
	3.5cc 3BA			1.25cc 1.25BA			

	26	27
	500	450
	2.5cc 3BA	died

R22 (HB R17)	12	14	15	16	18	19		21
	1810	1670	1720		1520	1550		1560
	6.5cc5BA			2cc6BA		4cc5.6BA		

	23	25	26	27
	1660	1650	1680	1550
			4.75cc7BA	died

R23 (KIDNEY R17)	12	14	15	18	19	21	23
	1250	1190	1200	1240	1120	1150	1220
	.3cc4BA			2cc5BA			

	25	26	28
	1220	1280	1030
		2cc4BA	killed

R24/

R24 (OSF R18)	22	23	31					
	1110	1060	1000					
	4cc5BA		died					
R25 (OSF R15)	10	12	14	15	16	17	18	
	620	640	600	613		590	600	
	1cc2BA	2cc1.8BA			2cc2BA			
	19	21	25	26	33			
	620	620	570	540	430			
			3.5cc4.5BA		died			
R26 (OSF R15)	10	12	26	34				
	550	590	530	510				
	.5cc1BA	3.5cc4.5BA		died				
R27 (OSF R17)	12	14	15	18	19	21	23	
	560	570	580	570	690	550	560	
	3.8cc3BA				2cc2.75BA			
	26	36						
	470	450						
	2cc2BA	died						
R28 (BILE R19)	24	26	36					
	520	490	480					
	3cc5BA		died					
R29 (H3)	129	130	136	138	144	153	171	178
	1420	1450	1370	1390		1350	1470	1513
	5SAS	5SAS	5SAS		5SAS			
	193	200	212	233	239	276	330	245
	1670	1500	1640	1900	1870	1800	1870	1820
	18	50						
	1670	?1920						
		killed						
R30 (P)	45	51	53					
	730		530	died				
	3.5cc4.9BA	4cc5.6BA						

R31/

R32 (P)

45	51	54
590		450
1.25cc	4cc	
1.75BA	5.6BA	killed

R33 (NOT WELL - TUMOUR - KILLED AS CONTROL).

R34 (WEIGHT 1700 - KILLED AS CONTROL).

R35 (O)

52	57
690	670
3cc1.5BA	killed, because dying.

R36 (O)

52	58
1040	900
3cc6BA	died

R37 (O)

52	58
920	850
4cc3BA	killed

R38 (ULCER R28)

52	58
1320	1020
2.5cc3.75BA	killed

R39 (P)

45	51	59
520		550
4.5cc	5cc	
6.3BA	7BA	killed.

R40 (KIDNEY R17)

12	14	15	18	19	21	23
1420	1290	1310	1190	1190	1270	1280
5cc4.5BA				.8cc2BA		

25	26	60
1250	1280	1340
	1.5cc3BA	KILLED

R41 (BILE R18)

22	23	60
1120	1120	1150
5cc5BA		killed

R42/

R42 (H)	123	124	129	130	131	136	138	144
	1400	1450	1470	1420		1350	1370	1400
	7SAS		5SAS	5SAS	5SAS	5SAS		5SAS
	151	152	153	155	178	193	200	212
	1400	1340	1350	1300	1220	1320	1350	1500
		5SAS						
	276	326	330	345	18	60		
	1850	1870	1820	1820	1690	1680		
						killed		

R43 (LIVER R17)	12	14	16	17	18	19	21	23
	2480	2370		2320	2370	2350	2340	2270
	2.5cc5BA		2cc2BA		5cc5BA			
	24	26	63					
	2280	2110	2400					
	3.5cc10BA		killed					

R44 (OSF R17)	12	14	15	16	18	19	21	23
	1880	1690	1730		1520	1670	1740	1780
	.8cc4.5BA			3cc7BA		3cc5.25BA		
	25	26	63					
	1770	1760	1750					
		2.5cc4BA	killed					

R45 (OSF R5)	163	170	177	184	199	212	251	281
	2140	2050	1910	1890	1890	1890	2320	2320
	5SAS	5SAS	5SAS		7B			
	295	326	345	18	63			
	2290	2290	2240	2190	2050			
					killed			

R46 (CONTROL)	59	64	
	2350	2230	
	2cc4.8BA	killed	

R47 (KIDNEY R28)	59	65	
	1140	1030	
	1cc 9BA	killed	

R48/

R48 (KIDNEY R28)	59	65
	1060	1100
	.7cc6BA	killed
R49 (CONTROL)	59	77
	1950	1640
	3cc7.2BA	killed
R50 (0)	52	78
	640	470
	2cc4BA	killed

The last point in technique which ought to be mentioned is, that, if the interval between injection exceeded 8 days a small desensitizing dose was given intravenously ½ hour before the full dose.

2. Alterations in Weights.

All the animals that died showed a marked and steady decrease in weight up to the time of death, with the exception of one animal which had been injected with a strain isolated for over 3 weeks prior to injection.

3. Influence of passage on the Virulence of the Organisms.

Of the freshly isolated organisms strain "G" appeared to be the most virulent. Its virulence was greatly increased by passage as was shown by the death of Rabbit 4 on the 3rd day after inoculation as compared with Rabbit 2, which although a slightly heavier rabbit received almost 5 times the quantity of the same organism prior to its passage.

4. The Influence of the Age of the Strain on the Virulence of the Organisms.

It has been noted also that the virulence of the organisms varies directly with the length of time which has elapsed since they have been isolated. In order/

order to illustrate this Rabbits 1 and 9, which were injected with strain "G", may be taken as examples. Rabbit 1 was inoculated with a week old strain and survived only 4 days, whereas Rabbit 9 which received the strain 3 weeks after isolation, survived until the 55th day.

Rabbit 8 which did not die after inoculation, received its injections from a strain over 3 weeks old.

5. Lesions and Symptoms produced.

The lesion and symptoms produced in the Rabbits of the above series are detailed in the table which follows:-

TABLE/

TABLE

Symptoms:	No. of Rabbit.	Gross Lesions.	Microscopic Findings.
Convulsions and head retraction.	R1 (G)	Small vegetations on mitral valve. Infarct of lung. Cloudy swelling of liver. Miliary abscesses of kidney.	Numerous small abscesses are seen in the cortex of the kidney - they show cocci.
Convulsion and head nodding movements.	R2 (G)		Kidney: Miliary abscesses containing cocci. Heart: Cusps look thickened and oedematous. No size of definite vegetations or inflammation.
	R4 (CSF R2)	Vegetations on Mitral valve.	These were confirmed microscopically and contained organisms.
	R5 (I)	Fatty degeneration of liver.	Abscess in the bone-marrow which contains Gram positive cocci.
Convulsions and head nodding	R6 (CSF R4)	Vegetation on tricuspic valve. Pyaemic abscesses of Kidney. Slight pleurisy. " ascites.	Vegetations are seen to be of the ulcerative type and show streptococci. Pyaemic abscesses are visible in the kidney.
Headnodding and convulsions.	R7 (J)	Vegetations on tricuspid valve.	The vegetations seen. Naked eye show streptococci.
Headnodding and convulsions.	R8 (CSF R1)	Pyaemic abscesses of kidney.	Tissues not taken for examination.

Symptoms:	No. of Rabbit.	Gross Lesions.	Microscopic Findings.
	R9 (G)	Vegetations on tricuspid valve. Infarct of lung. Pleurisy. Ascites.	Large no. of cocci in clumps in the vegetations.
	R29 (H)	Subcapsular mottling of Kidney.	Spleen shows increase of fibrous tissue.
	R42 (H)	Negative	Cloudy swelling of Kidney - no interstitial change.

6. Recovery of Organisms from the Inoculated Animals.

(a) Technique.

The technique employed in recovering the organisms from inoculated animals which have died or have been killed was the same in all cases and was as follows:-

I. CEREBRO-SPINAL FLUID.

The skin of the rabbit was reflected from the area over the cervical spine; the spine was cut through with a sharp knife and the cut area cauterised. A sterile capillary pipette was passed down the spinal canal and as much as possible of the Cerebro-Spinal Fluid withdrawn. This was now discharged into a tube of broth.

II. HEART BLOOD.

In order to obtain a specimen of heart blood the chest wall was dissected away in order to expose the anterior aspect of the heart. The right ventricle was now seared and ~~that~~ then the heart blood withdrawn through this area with a capillary pipette and discharged into a tube of broth.

The same technique was observed in obtaining bile and urine.

III. SOLID ORGANS.

A piece of the organ required was removed with a sharp knife. The piece taken was usually the size of a hazelnut. When it had been obtained, it was seared by holding it in the Bunsen flame and then placing it in a tube of broth. It was then broken up as much as possible by means of a sterile platinum needle.

It was found that the spleen was very difficult to disintegrate and this may account in part for the scanty growths obtained from that organ.

The strains derived from the tissues or body fluids of the inoculated animals were always plated out 3 consecutive times, before they were used for re-injection.

6. Source from which the Organisms have been regained.

Every effort was made to regain and identify streptococci from the animal tissues and body fluids. There is appended below a table which indicates for each rabbit in the series the sources from which the organisms have or have not been recovered, together with the number of days which had elapsed since the last injection.

A "0" indicates a negative finding, while those marked with a "-" were not investigated.

TABLE/

TABLE.

No. of Rabbit	Heart Blood	C. S. Fluid	Fl. fr. joint	Pericardial and pleuritic fluid.	No. of days.
1	0	+	-	-	3
2	0	+	-	-	3
4	0	+	-	-	2
5	+	+	-	-	1
6	0	0	-	-	7
7	0	+	0	-	7
8	0	+	-	-	3
9	0	+	0	0	3

		C.S.F.	Urine	Kidney	Bile	Spleen	Liver	
29	0	-	+	0	0	0	0	271
42	0	0	0	0	0	0	0	273

All cultures were incubated for a week before finally regarding them as negative.

An interesting point, which might be mentioned here, is the constancy of the head symptoms in this series in which Streptococcus Salivarius was the organism used throughout and associated with this is the constancy with which the organisms were recovered from the Cerebro-spinal Fluid, while the blood cultures were invariably negative inspite of the fact that very little cerebro-spinal fluid was obtained as compared with the 1-2ccs. of blood which was always easily procurable.

7. Pathology of the Lesions.

For convenience the descriptions of the pathological conditions have been relegated to part III of this thesis.

SERIES B.

1. Cultures employed.

Tubes of serum broth were inoculated from the stock tube of serum broth as previously mentioned. The cultures were again incubated for about 18 hours before being used for injection. The strain dealt with in this series had all been treated by passage.

As/

As these cultures were rather bulky they were first of all centrifuged and the supernatant fluid pipetted off. It might be well to note here that at the time those experiments were carried out a satisfactory centrifuge was not obtainable, with the result that the cultures were not of a satisfactory strength.

A film was made as before of the centrifuged serum broth culture before injection.

2. Alterations in Weights.

The alteration in weight in the rabbits of this series was negligible as might be expected since they showed but little untoward effect from the injections.

3. Influence of Passage on the Virulence of the Organisms.

The strain of organisms injected into Rabbits 11 and 12 was originally derived from the Cerebro-spinal fluid and was passaged twice. There was however no apparent increase in its virulence.

4. Lesions produced.

TABLE/

TABLE

No. of Rabbit	Gross Lesion	Microscopic Findings.
R10 (C.S.F R9) (G)	Vegetations on Mitral valve	Interstitial myocarditis in papillary muscle. Unfortunately the sections did not pass through the valve
R11 (C.S.F. R10) (C.S.F. R9) (G)	Atheroma.	
R12 (C.S.F. R10) (C.S.F. R9) (G)	Atheroma.	
R14 (C.S.F. R5) (I)	Negative	Insidious Rickets - Osteolysis.

5. Recovery of the Organisms from the Inoculated Animals.

The streptococci present in the animals at the time of death, were investigated in the same manner as described in the previous series. In this series however the intervals between the death of the animal and the date of the last injection were on the whole greater, the results obtained are evidenced in the following table.

No. of Rabbit	Heart Blood	C. S. Fluid	Joint Fluid	Urine	Bile	Interval since last injection.
10	0	-	-	-	-	6
11	0	0	0	-	-	10
12	0	0	0	-	-	10
14	0	0	-	0	0	15

SERIES C.

1. Cultures employed.

The organisms used in this series were grown on blood agar slopes. The slopes were prepared by adding 2 to 3 drops of defibrinated rabbits blood to 2ccs of melted agar, which had been allowed to cool to about 45°C. These tubes were then inoculated as in the previous series and placed in the incubator for about 18 hours. The growth was always found to be/

be abundant, and, on washing the colonies from the slopes with normal saline a uniformly smooth emulsion was obtained. The amount used varied from 0.9 to 9 blood agar slopes and in bulk varied from 0•3 to 6•5ccs.

2. Alterations in Weights.

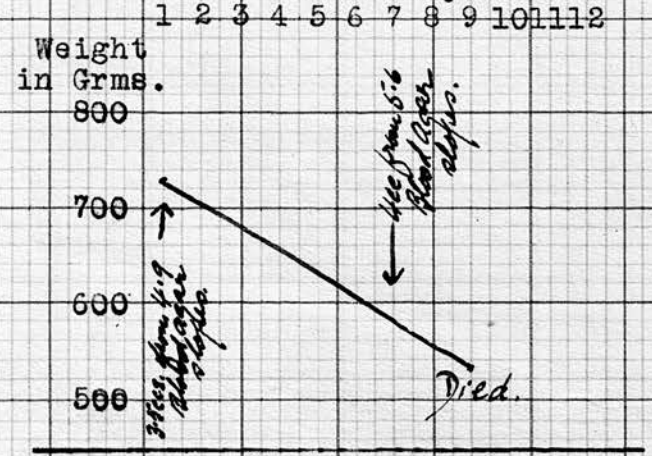
For the relationship between the weights of the animals during the course of injection, the amounts given and the intervals between them reference should be made to Table IV.

There is one rather interesting fact in this series, which has been emphasised by means of graphs, that is the extent to which the results may be swayed in such experiments as these by the natural resistance of the experimental animals. The graphs represent the alteration in weights of 3 rabbits, Rabbits 30, 32 and 39. These rabbits were all injected on the same day and with the same strain. Rabbit 39 was the smallest and youngest rabbit and it received a larger dose than rabbits 30 and 32, yet Rabbit 30 died after a rapid loss in weight, while Rabbit 39 actually gained weight.

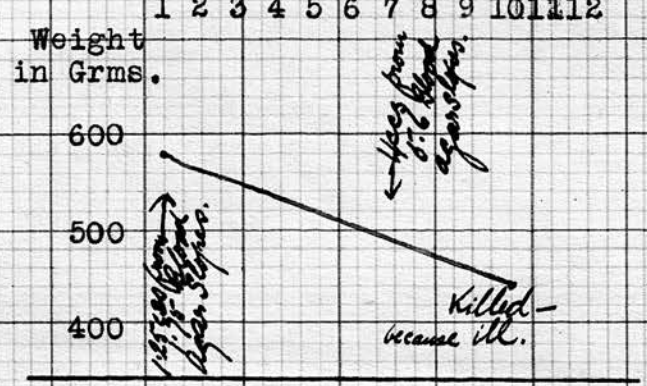
3. Influence of Passage on the Virulence of the Organisms.

Of the 23 animals employed, 4 were injected with a strain, freshly isolated from the patient, while 19 were/

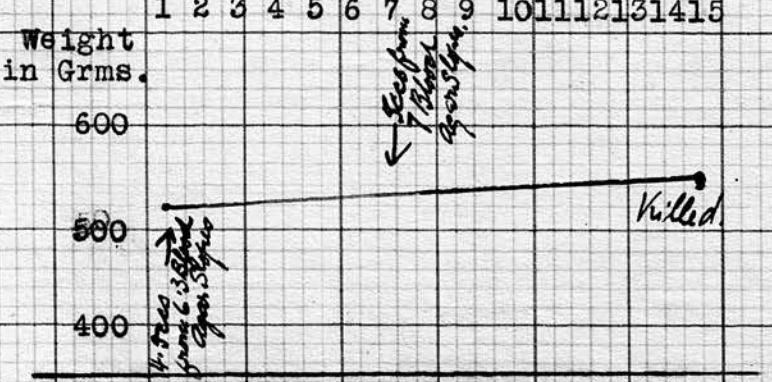
R.30.


No. of days.
1 2 3 4 5 6 7 8 9 10 11 12
Weight in Grms.
800
700
600
500
Died.

R.32.


No. of days.
1 2 3 4 5 6 7 8 9 10 11 12
Weight in Grms.
600
500
400
Killed - because ill.

R.39.


No. of days.
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Weight in Grms.
600
500
400
Killed.

were injected with organisms which has been passaged.

In this series 11 of the animals died while 12 of them were killed.

4. Lesions produced.

The lesions produced in this series are detailed in the following table:-

No. of Rabbit	Gross lesions.	Microscopic Findings.
R16 (C.S.F. R5)	Negative	Bone-marrow resembles that of an aplastic anaemia. Spleen: contains a tremendous amount of haemosiderin. Fatty degeneration of myocardium.
R17 (L)	2 small vegetations. Bronchiapneumonia.	The vegetations show only a slight proliferation of connective tissue. Not ulcerative.
R18 Urine (R17)	Negative	Cloudy swelling of Kidney. Fairly marked osteolysis in the long bones.
R19 (Urine R17)	Negative	Slight osteolysis in the long bones.
R20	Negative	Increase generally in the connective tissue of the spleen.

R21/

No. of Rabbit	Gross Lesions	Microscopic Findings.
R21 (Liver R17)	Pneumonia	General increase in connective tissue in the spleen.
R22 (H.B.R. 17)	Spleen enlarged	Broncho-pneumonia. Great congestion of myocardium.
R23 (Kidney R17)	Spleen enlarged	Osteolysis in long bones - also Fibrosis of Cartilage
R24 (C.S.F. R18)	Negative	Negative
R25 (C.S.F. R15)	Slight pleurisy and Ascites.	Commencing fibrosis in the kidney
R26 (C.S.F. R15)	Peritonitis. Spleen atrophied.	Broncho-pneumonia. Kidney: cloudy swelling. Spleen: inflammatory:- soft and spongy.
R27 (C.S.F. R17)	Negative	Negative
R28 (Bile R19)	Ulcers in stomach wall	Gastric ulcers with Gram positive cocci related to them. Osteolysis of long bones.

R30/

No. of Rabbit	Gross Lesions	Microscopic Findings.
R30 (P)	Negative	Osteolysis of long bones. Kidney: commencing fibrosis.
R38 (Ulcer R28)	Negative	Granular and fatty degeneration of the kidney. Pleurisy and lobar pneumonia.
R39 (P)	Infarct of lung.	Cloudy swelling and granular degeneration of the kidney.
R40 (Kidney R17)	Negative	Cloudy swelling and congestion of kidney.
R41 (Bile R18)	Emphysema.	Heart: Proliferation at root of aortic cusp with a calcareous plaque. Myocardium not fibrosed. Kidney: cloudy swelling and congestion. Spleen: Congestion. Lymphoid tissue increased.
R43 (Liver R1)	Negative	Area of early interstitial fibrosis in the kidney. Spleen: contains a large amount of pigment.
R44 (C.S.F. R17)	Negative	Kidney: cloudy swelling and congestion
R4 (Kidney R28	Negative	Congestion and oedema of kidney. Spleen: appears necrotic.
R48 (kidney R28)	Nodular thickenings in lung.	Broncho pneumonia

5. Recovery of Organisms from the Inoculated Animals.

The organisms recovered and the sources from whence they were obtained as the result of the technique described are represented in the table below.

No. of Rabbit	Heart Blood	C. S. Fluid	Spleen	Liver	Urine	Kidney	Bile	Interval in days.
16	0	0	0	0	-	-	-	10
17	+	+	-	+	+	+	-	20
18	-	+	0	-	-	0	0	3
19	0	+	0	-	+	0	-	2
20	0	0	0	0	0	+	+	6
21	+	+	0	+	+	0	+	1
22	-	0	-	+	-	+	0	1
23	+	0	0	+	-	+	+	2
24	0	+	0	0	0	0	0	9
25	+	0	0	0	+	+	+	8
26	0	0	-	+	+	0	0	9
27	0	-	0	0	+	0	0	10
28	0	+	0	0	+	+	0	12
30	+	+	+	+	+	+	+	2
32	0	0	0	0	+	+	0	3
38	+	+	-	-	-	+	-	6
39	-	-	-	-	+	+	+	8
41	-	-	-	-	+	+	+	38
40	0	0	0	0	0	+	-	34
43	0	0	0	0	0	0	0	39
44	0	0	0	0	0	0	0	37
47	-	-	-	+	-	+	+	5
48	-	-	+	-	+	+	-	6

In this series the Streptococcus Faecalis was used throughout. An interesting point occurs in comparing this table with Table I, and that is that, whereas salivarius may be constantly recovered from the cerebro-spinal fluid and where its presence is associated with nerve symptoms, especially convulsions, choreic movements, etc., S. Faecalis was only recovered from the cerebro-spinal fluid in less than half the cases and was never associated with any nerve symptoms. Whether or not this is due to a natural immunity resulting from the presence of the S. Faecalis as a natural inhabitant of the intestinal tract of the rabbit or not is a question open to speculation.

It was noted also in this series that the organisms could be recovered in pure culture as long as 38 days after the last injection.

In rabbit 18, they were even present in the blood 20 days after injection which was exceptional.

The streptococci showed the greatest persistance in the bile, urine and kidney, the kidney tissue showing it most markedly.

SERIES D.

1. Cultures employed.

2 rabbits composed this series. The rabbits were injected partly with organisms grown on serum agar/

agar slopes and partly with organisms grown in serum broth.

2. Alterations.

The first rabbit (R13), showed improvement after the injections were completed and only lost weight markedly long after its last injection.

The other Rabbit (R45) in contrast to this showed a marked decrease in weight after the 1st and 2nd injections. After it became actively immunised it gained weight over a considerable period and then once more gained weight.

3. Lesions produced.

Lesions produced are detailed in the following table:-

No. of Rabbit	Gross Lesions.	Microscopical Findings.
R13 (C.S.F. R4) (C.S.F. R2) (G)	Negative	Acute Nephritis.
R45 (C.S.F. R5) (I)	Negative	Cloudy swelling of Kidney.

4.

With regard to the recovery of organisms from the tissues and body fluids the results are negative as is shown by the following table:-

No. of Rabbit	Heart Blood	C. S. Fluid	Urine	Bile	Spleen	Kidney	Liver	Interval since last injection in days.
R13	0	0	0	0	-	-	-	84
R45	0	0	0	0	0	0	0	222

CONTROL RABBITS.

5 Control Rabbits were carefully examined which varied in weight from 1700-2350 grammes.

3 were killed without being treated in any way - while 2 had previously received injections of normal saline washings from 5 to 7 blood agar slopes.

The tissues were investigated for pathological lesions the only one present was a slight degree of cloudy swelling in the kidneys of Rabbit 46, otherwise the tissues presented a normal histology.

The tissues and body fluids were also investigated for the presence of streptococci and the results are tabulated below:-

TABLE/

No. of Rabbit	Heart Blood	C. S. Fluid	Urine	Bile	Kidney	Liver	Spleen
R31	0	0	0	0	0	0	0
R33	0	0	0	0	0	-	-
R34	0	0	-	0	0	0	0
R46	0	0	-	0	0	0	0
R49	0	0	-	0	0	0	0

HAEMOLYTIC STRAIN.

In order to test and compare the effects produced by a haemolytic Streptococcus, a strain was employed which had been isolated from a case of osteomyelitis.

It belonged to the Infrequens type of Holman and was lethal to mice within 12 hours. The organisms were grown on blood agar slopes and were injected into 4 rabbits.

Its effect upon the weights of the animals will be seen in the "weight table" under the designation of strain "O". The animals became very ill and went off their food to a much more marked extent than any animals injected with S. Viridans. Only one injection was given as the animals were too ill to re-inject.

They/

They presented lesions detailed in the following table:-

No. of Rabbit	Gross Lesions	Microscopical Findings.
R35 "O"	Myocardial abscesses	Brain: Abscess in brain containing numerous cocci in pair and chains.
R35 "O"	Myocardial abscesses	Large inflammatory focus about 1" above the joint surface of the femur. The tissue was very hard to cut and unfortunately Gram section could not be obtained on this account.
R37	Myocardial abscesses	Joint: Pus in the joint and damage to the articular cartilage.
R50	negative	negative

It is interesting to note that this was the only strain which produced a suppurative lesion in the brain and in the joint, as will be detailed later under the Pathological description.

An attempt was made to recover the organisms from the body fluids and tissues, the results of which are appended below:-

No. of Rabbit	Heart Blood	C.S.F.	Bile	Spleen	Liver	Kidney	Urine	Interval since Injection.
R35	0	-	0	0	0	+	+	5
R36	0	-	0	0	0	0	-	6
R37	+	-	-	+	-	-	-	6
R50	-	-	-	-	-	-	-	26

CONCLUSIONS.

1. There is no definite uniformity in the alteration in weight of the injected animals.

2. That the virulence of Streptococci is increased by passage and decrease by repeated subculturing, but as a whole their virulence is extremely low.

3. In a very short time it was found impossible to recover organisms from the heart blood, while they persisted in the cerebro-spinal fluid.

4. That they appear to be eliminated very slowly by the kidneys and have been regained from the urine long periods after the other body fluids proved negative.

5. That a large part in the determination of the effects of the injections appears to depend upon some inherent factor in the individual rabbit.

PART III.

NOTES ON SYMPTOMS AND PATHOLOGY OF LESIONS PRODUCED.

Out of 70 Rabbits injected with Streptococci from cases of Pyorrhoea Alveolaris, seven showed distinct endocardial lesions. Four of these occurred on the mitral valve, while 3 occurred on the tricuspid (see photographs 1, 2, 3 and 4.

It is interesting to note that six out of eighteen animals injected with S. Salivarius developed endocarditis, while only one of the eighteen rabbits injected with S. Faecalis showed an endocarditis, which occurred as two small vegetations on the tricuspid valve in Rabbit 17.

All the vegetations, with one exception (as the specimen was mounted intact), were examined microscopically. The valvular lesions in all cases injected with S. Salivarius showed superficial necrosis and erosion of the endothelium, which was usually markedly proliferated. A variable amount of organised blood clot containing Streptococci was as a rule adherent to this. (See microphotographs 5 and 6).

In one case, Rabbit 2, there was no definite vegetation/

vegetation, but microscopically one could detect a thickening of the cusps, due chiefly to a marked oedema separating the connective tissue elements.

No control animal showed any trace of endocarditis.

NOTES ON THE LITERATURE ON EXPERIMENTAL ENDOCARDITIS.

A. Historical.

Early workers found that they were unable to produce endocarditis without imparing the vitality of the cusps before the injection of micro-organisms.

Wyssokowitsch[30], Fraenkel and Saenger[31], and Weichselbaum[32], found that an intravenous injection of organisms alone could not produce endocardial lesions.

About the same time (1887), Prudden[33] applied caustics to the valves before the injection of organisms.

Ribbert[34] (in1886) tried injecting an emulsion of potato along with staphylococci, as he thought the particles of potato would mechanically damage the valves and form a suitable nidus for the organisms.

Dreschfeld[35], however, was the first (in1887) to produce endocarditis by injecting streptococci alone.

B. Recent Work on Endocarditis.

Since/

Since then numerous observations of a similar nature have been described by workers such as Shaw[36], Libman and Cellar[37].

Rosmon[38], by injecting very large quantities of cocci derived from patients suffering from subacute or chronic endocarditis was able to produce endocardial lesionsin 84% of his rabbits.

Henrici[39] found that 9% of the rabbits he inoculated with non-haemolytic strains of Streptococci developed lesions of the heart valves.

Poynton and Paine[40] were sure that their work had firmly established the relationship between rheumatism and both simple and malignant endocarditis.

Moody[41] isolated Streptococci from cases of Chronic Alveolar Abscess and injected them into 178 rabbits. He allowed the animals to live for 5 - 8 days. Only in one case he found well developed vegetations. The organisms used by him were of the Viridans variety.

Hartzell and Henrici[4] while working with Streptococci derived from cases of Pyorrhoea Alveolaris and of Apical Abscesses were only able to produce sub-endothelial haemorrhages in a series of 24 animals.

An interesting point is noted by Andrewes and Horder[21] during the examination of 21 cases of malignant endocarditis due to streptococci. To their 21 cases they added 3 reported on by Gordon. Of these

24/

24 cases, 11 were found to be due to S. Salivarius, 6 to S. Anginosus, 4 to S. Faecalis, 2 to S. pyogenes, 1 to the pneumococcus. The very large proportion associated with S. Salivarius conforms with the findings in this research.

II. KIDNEY LESIONS.

1. Interstitial Changes.
2. Abscesses.

These two changes were the most outstanding of all the kidney lesions which occurred in the series of animals.

Almost all the rabbits showed a degree of cloudy swelling. In the tables of Pathological findings, cloudy swelling is only mentioned when it is marked, otherwise, it is omitted. No weight can be placed on this disorder, as not only is it a condition described in the literature as occurring in normal rabbits, but it occurred in Rabbit 46, a control rabbit in this series of experimental animals. No further mention will therefore be made of it..

I. INTERSTITIAL NEPHRITIS.

The descriptions of the cases of interstitial changes occurring in the Kidney are given below.

The least marked changes are given first.

A. Kidney of Rabbit 30.

There was no gross lesion.

Microscopically: The kidney was markedly congested. In the subcapsular region of the cortex the peri-glomerular spaces had disappeared and fibrosis had commenced in some of the glomerular tufts.

The glomeruli in the medullary zone of the cortex appeared fairly healthy. In this region there was a tendency to dilatation of the convoluted tubules, these occurred in clusters and in their vicinity the cells were very granular and there was a commencing fibrosis.

Most of the dilated tubules contained casts, while there were blood casts in some of the straight tubules. An inflammatory focus was present in the kidney. Streptococci were visible in all parts of the section.

B. Kidney of Rabbit 25.

There was no gross lesion. A similar condition was seen microscopically in the kidney of this rabbit.

Microscopically/

Microscopically:

The cortex was slightly diminished. The space between Bowman's capsule and the glomerular tuft was absent. The glomeruli were very congested and there was evidence of an early fibrotic change in them. In some places the glomeruli appeared to adhere to the capsule. There was a general congestion which was especially marked in the cortex.

C. Kidney of Rabbit 17.

There were no gross lesions.

Microscopically:

The sections from this kidney showed an appearance akin to that of an overwhelming infection. The glomeruli were swollen and occupied the whole of Bowman's capsule in some parts, in others they appeared fairly normal. There was an increase of the connective tissue in the medulla.

D. Kidney of Rabbit 43.

No gross lesions were found in this kidney.

Microscopically:

This kidney showed an area of interstitial nephritis, with destruction of the tubules, only relics of which were left. This area did not extend to the cortex.

The/

The four sections described above only showed very early forms of interstitial nephritis.

In the following two rabbits the interstitial changes were very much more marked.

E. Kidney of Rabbit 12.

Microscopically:

There were two areas of well marked interstitial nephritis in the sections taken from the kidney if this rabbit. Both areas extended to the cortex and can be seen in the micro photographs (Nos. 7 and 8). At the part where the affected area reached the surface a distinct depression could be seen.

There was no difference in the areas found in Rabbit 12 and those found in Rabbit 29, so that the description of them in Rabbit 29, will serve at the same time to describe those in Rabbit 12.

F. Kidney of Rabbit 29.

Microscopically:

The section presented four areas of fibrosis, one of which did not extend to the capsule of the kidney, but only midway through the cortex. The other three took the form of wedges with their bases to the capsule (see microphotograph 9).

At the point where the base met the capsule there was a distinct depression.

In/

In the cortex there was a variety of stages in the process of fibrous tissue formation. Some parts were almost normal, and from this almost all degres up to complete sclerosis could be found.

In all the fibrous areas the glomeruli were more or less normal, and only in the more advanced fibrous areas were the glomeruli beginning to change; there appeared to be complete occlusion of the afferent duct and no communication between tuft and tubule.

The condition present viewed generally did not strike one as being a primary glomerular condition. The earliest change seemed to be a proliferation of the interstitial cells, which replaced the convoluted tubules. There probably was a very slight degree of interstitial proliferation, and associated with it, or rather, preceeding it there was dilatation of the convoluted tubules, which showed marked changes in their cells. The cells formed a degenerate type of epithelium, which was becoming stratified, or which was disappearing and in several places retention cysts had formed. The amount of connective tissue related to the tubules was not marked. In some parts the tubuled seemed to be disappearing without these preliminary changes.

All processes suggested that the primary changes occurred in the tubules and interstitial tissue and that the fibrosis of the glomeruli occurred at a much/

much later period. In the glomeruli one noted a gradual sclerosis until there was only a knot of fibrous tissue left.

The fibrous tissue was found to be vascular and that there was a very marked fibrosis about Bowman's capsule.

There were various groups of small round cells scattered irregularly throughout the cortex and also in the medulla.

The congestion was definitely patchy in this section.

At the base of the wedges the fibrous tissue seemed to extend under the capsule and also into the kidney tissue on either side. The apex of the fibrous area extended down along the straight and collecting tubules to the medulla. There was also a definite increase in the interstitial tissue in the medulla and consequently a diminution in straight tubules.

LITERATURE ON INTERSTITIAL NEPHRITIS.

Henrici and Hartzell[4] did not accept any lesions in the kidney, as due to the injection of streptococci, if fibrosis or dilatation of the tubules occurred, as these animals were only permitted to live for 10 days.

Moody/

Moody[41] found no changes of this nature in 178 rabbits injected with streptococci derived from cases of Pyorrhoea Alveolaris.

A FEW NOTES ON THE INTERSTITIAL CHANGES PRODUCED IN THIS RESEARCH.

One of the cases was produced by S. Salivarius, while 5 rabbits injected with S. Faecalis developed interstitial lesions.

It is interesting to note the Streptococci of the faecalis variety were recovered from the Urine of Rabbit 29, 271 days after its last injection.

Unfortunately this was the only case of this kind, otherwise some interesting conclusions might have been drawn, but as it is, it only stands as an interesting and suggestive case.

II. ABSCESSES.

Rabbits 1, 2, 6 and 8 showed small abscesses in the cortex of the kidney.

A. Abscesses in Rabbit 1 and Rabbit 6.

There were seen dotted over the surface at post mortem/

mortem examination of these rabbits.

Microscopically:

Abscesses were seen scattered throughout the cortex. Clumps of cocci were seen in the centre and were surrounded by a lot of cell debris. A profuse polymorph infiltration was found in relation to these areas.

The abscesses were localised and tended to spread along the interlubular tissue spaces.

B. Abscesses in Rabbit 2.

These were not seen at post mortem examination of the Rabbit.

Microscopically:

There were numerous abscesses in the kidney; they were related to glomerular tufts and interlubular vessels (see microphotograph 10).

Clumps of cocci were found in them. A definite area of necrosis surrounded the organisms. The chief reaction in the glomerular tuft appeared to be at the point of entrance of the blood vessels. Here organismal emboli could be seen with the lumen of the blood vessels. The glomeruli in some places were destroyed by the abscesses and replaced by inflammatory cells.

The abscesses had a very wide distribution.

The capillaries were very congested and distended.

There/

There was a proliferation of the connective tissue cells about the glomerular tufts. In many cases the glomeruli had become adherent to Bowman's capsule, which had proliferated.

There was also a proliferation of connective tissue within the tuft. Protoplasmic granules were seen in the tubules. The cells did not stain well and could not be distinguished. Some of the nuclei were disappearing, while others stained intensely and showed an accumulation of chromatin round the periphery.

C. Kidney of Rabbit 8.

Abscesses seen at post mortem, they were not examined microscopically.

The urine and kidney tissues were not examined culturally for organisms in any of these Rabbits.

In this series of experiments, abscesses were only found in animals which had been injected with S. Salivarius

---

LITERATURE ON ABSCESSES PRODUCED IN THE KIDNEY BY STREPTOCOCCI.

Hartzell and Henrici[4] found that S. Viridans caused abscess formation in the kidney and that these usually occurred in the cortex, but occasionally also in the medulla.

Henrici[39] when working alone, also found abscesses in the kidney after the inoculation of rabbits with streptococci.

Similar results were obtained by Le Count and Jackson.[42]

They all found, as my observations ~~tried~~ tend to show that the abscesses were more frequently present in the cortex and almost always occupied a perivascular position.

Moody[41] did not describe kidney lesions of this nature in his work on Streptococci from cases of Chronic Alveolar Abscess. He merely obtained small haemorrhagic foci usually situated beneath the capsule and scattered through the cortex.

III. EARLY STAGE OF RICKETS.

Six rabbits out of 40 inoculated with streptococci of the viridans type showed a slight degree of osteolysis - none of them showed a more advanced stage in rickets.

It/

It is interesting to note that none of the control rabbits showed the condition, and, although slight it occurred in a considerable number of the animals experimented on.

The condition might be due to a stimulus arising from the injection of Streptococci. On the other hand, is a week to 15 days sufficient for such a lesion to develop in? It is difficult to say and much work would be required to clear up this point.

In all the cases the change was in the diaphyseal portion of the long bones. There was no change in the epiphysis (see microphotographs 11, 12 and 13).

Only one case occurred in an animal injected with S. Salivarius, all the others occurring in animals which had been injected with S. Faecalis.

I have not encountered the description of such lesions, as the result of Streptococcal inoculations, in any of the literature. Even Aschoff[43] does not mention infection as a possible cause of rickets.

I have merely drawn attention to these lesions. As I have known rickets does not occur spontaneously in rabbit, I do not wish to make a definite statement and ascribe it to the Streptococcal inoculations.

IV. OTHER LESIONS.

There were a few lesions which occurred in only one or two animals, but which I consider are worthy of note.

I. APLASTIC ANAEMIA (?)

The appearances of an aplastic anaemia were shown in the bone marrow of the femur and tibia, and, also in the large amount of haemosiderin found in the spleen. Unfortunately a blood film was not examined.

A. Bone Marrow.

Microscopically:

There was practically nothing of a haemogenetic type left in the ~~kidney~~ marrow. In a field of the microscope one could only detect one or two cells with a granular protoplasm.

There were a number of cells present which probably belonged to the lymphocytic type, but there were no groups of lymphocytes, no nucleated reds or mega-kariocytes.

The fat cells were moderate in number. There were a few connective tissue cells with spindle shaped bodies. The matrix was occupied by a fine fibrous network and this was the prevailing structure. Here/

Here and there small islands of haemogenetic cells persisted, in which the chief cells were myelocytes, also a few polymorphs, and occasionally a nucleated red blood cell. Even these were showing atrophy. There was no deposite of pigment and the texture resembled that of the synovial fringes. (see microphotograph 14).

B. Spleen.

I. STAINED WITH HAEMATOXYLIN AND EOSIN.

Microscopically:

It could be seen that by this method of staining the masses of deposit were observed forming large granules in some cells, smaller granules in others, and yet in others a diffuse pigmentation of the whole cell substance. The large granules had been engulfed by the endothelial phagocytes, some of which were observed to contain red blood cells, which still stained with eosin.

II. PRUSSIAN BLUE SECTION.

There was a tremendous amount of haemosiderin seen in this section, but it was not in the usual granular form. The blue coloration was suffusing and pigmenting the whole. Little masses of debris were lying in the sinuses and were similarly stained blue. The Malpighian bodies were almost altogether free from deposit. (See microphotograph 15).

The/

The most careful application of the Prussian Blue stain could not reveal any haemosiderin in either liver or kidney.

II. GASTRIC ULCERS.

These occurred in one case. Streptococci conforming in type to those injected, were regained from the ulcers.

The ulcers were very numerous and were confined to the stomach. At post mortem examination - no thickening of the peritoneum overlying the ulcers could be made out.

Microscopically:

There was a large haemorrhage on to the mucous surface of the stomach and also a fibrinous exudate on it. The stomach wall showed necrosis at the site of the ulcers. There was marked congestion of the mucous surface throughout.

No glands were left in relation to the ulcers, here there was only necrotic tissue and an infiltration with inflammatory cells. There was no peritonitis. Diplococci and a few cocci arranged in chains were seen in relation to it. They extended down to the muscular coat.

The most noteworthy work on this subject, is that/

that by Rosenow who produced gastric ulcers with great regularity in animals.

III. ATHEROMA OF THE AORTA.

A very marked atheroma of the aorta was found in Rabbit 12 (see photograph 16), while in Rabbit 11 there were only two small atheromatous patches to be seen.

These findings were confirmed microscopically, but little stress can be laid on them, since no streptococci were found in relation to them. Atheroma is a fairly common spontaneous lesion in rabbits. The change found in these two rabbits was confined to the intima, which was thickened.

There was no change in the media.

Knotz[45] drew attention to the occurrence of a thickening of the intima of the aortic arch in Rabbits repeatedly injected with Streptococci, whose virulence was low. In a paper published some years later, he gives further reports on the condition. Henrici[39] also described the condition in 4 animals.

IV. ARTHRITIS.

The only case of arthritis occurring in these animals/

animals was in an animal injected with a haemolytic strain (see microphotographs 17 and 18).

It was mainly in the hope of producing arthritis by the injection of Streptococci from cases of pyorrhoea alveolaris that this work was undertaken.

No other lesions obtained require special mention, since they were not striking enough in character.

SYMPTOMS.

CHOREIFORM MOVEMENTS.

Choreiform movements were observed in 5 of the inoculated animals; they took the form of headnodding movements and as the animal became worse, of incoordinated convulsive movements, which were greatly aggravated by handling the animal.

When one of those rabbits was laid on its side, a long time elapsed, before the upright posture was resumed. In one or two cases the animals were completely unable to do so. Later these rabbits became very violent and threw their bodies against the cages to such a distressing degree that 3 of them had to be killed. The remaining two showed the symptoms to a much less extent.

All/

All the rabbits showing these movements were inoculated with S. Salivarius and the S. Salivarius was recovered from the cerebro-spinal fluid of all except one, in which there was an interval of seven days between the last inoculation and its death.

It is also worthy of note that Streptococci were not recovered from the heart blood in any of these cases.

Similar cases have been recorded in the literature, but they are very rare.

The first writer to report such a condition was Professor Beattie[46], who found it in one animal on the 2nd day after intravenous injection of streptococci.

Floyd[47] in an extensive research in the relationship of mouth streptococci to chorea failed to obtain any conclusive results.

CONCLUSIONS.

1. Arthritic changes were entirely absent in all cases, with one exception, where the Streptococcus was a haemolytic one derived from a case of osteomyelitis.

2. That endocarditis could be produced by the injection of streptococci alone and that in all cases it was the S. Salivarius that produced this lesion.

3. That definite kidney lesions mainly of interstitial type occurred and in these cases it was always the streptococcus faecalis which was the causal organism. The streptococci could be recovered from the urine over a prolonged period after injection, thus demonstrating its prolonged action upon the kidney during the process of elimination.

4. The only suppurative lesions which were found occurred in the kidney and in the heart, in all cases these focal infections were due to S. Salivarius, thus demonstrating its greater tendency to localise and produce pus, than the S. Faecalis.

5./

5. That definite choreiform movements can be produced by the injection of streptococci recovered from cases of oral sepsis.

6. That a large number of other pathological lesions were found which may indicate that Streptococci may be responsible for many diverse conditions in the human subject.

GENERAL SUMMARY.

1. That in all cases of Pyorrhoea Alveolaris Streptococci could be recovered in pure culture.

2. That those Streptococci were always of the viridans group.

3. That in animals the subjects of active lesion, the blood culture was very frequently negative, demonstrating the difficulty in the use of blood culture for diagnostic purposes in the human subject.

4. That a great diversity of pathological lesions could be produced and these were not of the same type as the co-existing lesions in the patients from which the organisms were recovered.

5. That the S. Salivarius showed special affinity for the heart valves and for localising and producing focal infections, while S. Faecalis tended to produce non-suppurative lesion of a diffuse type, which appeared to be due more to the persistence of the toxins rather than the actual organisms.

6./

6. That in chronic kidney lesions, organisms could be regained from the urine over prolonged periods which indicated an important point in the investigation of similar conditions in the human subject.

I wish here to express my thanks to Professor Lorrain Smith and Professor Mackie for their assistance and encouragement throughout this research.

My indebtedness is also due to Professor Bramwell, Dr Chalmers Watson and Mr Gibbs, for the facilities given me in obtaining material and data from patients under their charge.

BIBLIOGRAPHY.

1. Euler: Kl. Wochenschr, 1923, 34, 1605.

2. Bertrand & Valadier: Compt. Rend. de la Soc. de Biologie, 1913, 75, 432.

3. Gilmer & Moody: J. Am. Med. Assoc. Dec. 5, 1914, 2023.

4. Hartzell & Henrici: Journ. Am. Med. Assoc. 1915, 64, 1055.

5. Seitz: Centrabl. f. Bakt. Abt. I.Orig., 1922, 89, 135.

6. Kinsella & Swift: Journ. Exp. Med. 1917, 25, 877.

7. Howell: Journ. Inf. Dis. 1918, 22, 244.

8. Aschner: Journ. Inf. Dis. 1917, 21, 409.

9. BesredRa: Am. de l'Inst. de Pasteur, 1904, 18, 363 and 373.

10. Floyd & Wolbach: Journ. med. Res. 1913, 14, 29, 493.

11. Hiss: Journ. Exp. Med. 1905, 7, 547.

12. Krumwiede & Valentine: Journ. Inf. Dis. 1916, 19, 760.

13. Swift & Thro: Arch. Inf. Med. 1911, 7, 24.

14/

14. Wolbach: Journ. Med. Res. 1914, 29, 493.

15. Havens: Journ. Inf. Dis. 1919, 25, 315.

16. Kligler: Journ. Inf. Dis. 1915, 16, 329.

17. Smith & Brown: Journ. Med. Res. 1915, 31, 455.

18. Barnes: Journ. Inf. Dis. 1919, 24, 47.

19. Holman: Journ. Med. Res., 1916, 34, 377.

20. Gordon: Res. Medical Officer to Local Govt. Bd. Gt. Britain, 1902-3, 32, 421, 1903-4, 33, 388.

21. Andrewes & Horder: Lancet, 1906, 2, 708.

22. Neufield: Zeitschr. f. Hyg. 1901.

23. Libman & Rosenthal: Proc. N.Y. Path. Soc., March, 1908.

24. Sekiguchi: Journ. Inf. Dis. 1917, 21, 475.

25. Schottmüller: Munch. Med. Wochenschr. 1903, 1, 849; 909.

26. Lyall: Journ. Med. Res. 1914, 30, 515.

27. Cowan: Brit. Journ. of Exp. Path. 1922, 3, 187.

28. Kendall, Day, Walker & Ryan: Journ. Inf. Dis. 1919, 25, 205.

29. Ainley Walker: Proc. Roy. Soc. Series B., 1912, 85, 400.

30./

30. Wyssokowitsch: Virchow's Arch. f. path. Anat. 1886, 103, 301.

31. Fraenkel & Saenger: Virch. Arch. f. path. Anat. 1886, 103, 333.

32. Weichselbaum: Centralbl. f. Bakt. 1887, 108, 286.

33. Prudden: Am. Journ. Med. Sc. 1887, 2, 207.

34. Ribbert: Fortschr. d. Med., 1886, 41.

35. Dreschfeld: Brit. Med. Journ., 1887, 2, 887.

36. Shaw: Journ. of Path. & Bact. 1904, 9, 158.

37. Libman & Cellar: Am. Journ. Med. Sc. 1910, 140, 576.

38. Rosenow: Journ. Inf. Dis., 1912, 11, 210.

39. Henrici: Journ. Inf. Dis., 1916, 19, 572.

40. Poynton & Paine: Researches on Rheumatism, 1914.

41. Moody: Journ. Inf. Dis., 1916, 19, 514.

42. Le Count & Jackson: Journ. Inf. Dis., 1914, 15, 389.

43. Aschoff: Path. Anat., Jena, 1923.

44. Rosenow: Journ. Inf. Dis., 1916, 19, 333.

45. Klotz: Brit. Med. Journ. 1906, 2, 1767.

46. Beattie: Brit. Med. Journ. 1904, 11.

47. Floyd: Journ. Med. Res. 1919-20, 41, 467.

Fig.1. Small vegetations on mitral valve of Rabbit 10.

Fig.2 Large vegetations on tricuspid valve of Rabbit 9.

Fig.3 Large vegetations on tricuspid valve of Rabbit 6.

Fig.4 Enlargement of Fig.3. Showing vegetations on tricuspid valve of Rabbit 6.

Fig.5 Vegetation on tricuspid valve of Rabbit 6. The masses of organisms can even be made out in this low power photograph.

X20

Fig.6 Clumps of organisms in the vegetations on the tricuspid valve of Rabbit 6.
X1000

Fig.7 Showing one of the areas of interstitial nephritis in Rabbit 12.

X60

Fig.8 Showing the other area of interstitial nephritis in Rabbit 12.

X60

Fig.9 Showing an area of interstitial nephritis in Rabbit 29.

X30

Fig.10 Abscesses in the kidney of Rabbit 2. They are chiefly related to the glomeruli.

X30

Fig.11 Osteolysis in the diaphysis of the femur in Rabbit 18.
X30

Fig.12 Part of the femur from Rabbit 18, but also showing the diaphyseal cartilage.
X30

Fig.13 Also showing osteolysis but of a lesser degree in Rabbit 19.

X30

Fig.14 Showing the bone marrow in Rabbit 16, in a case of ? Aplastic Anaemia.

X30

Fig.15 Spleen from Rabbit 16 stained with Prussian Blue, showing a very heavy deposit of haemosiderin.

X50

Fig.16 Showing atheromatous patches in the aorta of Rabbit 12.

Fig.17 Showing abscesses in the knee joint of Rabbit 37 injected with a haemolytic streptococcus.

X30

Fig.18 From the same joint as Fig.17, showing destruction of the articular cartilage.

X30